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6. AUTHORS
Liju Yang

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The views, opinions and/or findings contained in this report are those of the author(s) and should not contrued as an official Department of the Army position, policy or decision, unless so designated by other documentation.

14. ABSTRACT
The Specific Aims of the project were to investigate: 1) the effect of single-walled carbon nanotubes (SWNTs) with different surface groups on the inactivation of B. anthracis spores; 2) the effect of SWNTs coupled with near-infrared (NIR) on B. anthracis spores; 3) the effect of SWNTs in combination with antimicrobial chemicals on inactivation of B. anthracis spores; 4) the effect of CNTs coated surfaces on the inactivation of B. anthracis spores; 5) the mechanisms of the inactivation effects of SWNTs to B. anthracis spores. Results of findings and their significance: We found that: 1) SWNTs were effective in inactivating B. anthracis cells, but not effective to B

15. SUBJECT TERMS
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a. REPORT	b. ABSTRACT	c. THIS PAGE			Liju Yang
UU	UU	UU	UU		19b. TELEPHONE NUMBER 919-530-6704

Report Title

Final Report: (Life Science Division/Biochemistry) Inactivation of Bacillus Anthracis Spores Using Carbon Nanotubes

ABSTRACT

The Specific Aims of the project were to investigate: 1) the effect of single-walled carbon nanotubes (SWNTs) with different surface groups on the inactivation of B. anthracis spores; 2) the effect of SWNTs coupled with near-infrared (NIR) on B. anthracis spores; 3) the effect of SWNTs in combination with antimicrobial chemicals on inactivation of B. anthracis spores; 4) the effect of CNTs coated surfaces on the inactivation of B. anthracis spores; 5) the mechanisms of the inactivation effects of SWNTs to B. anthracis spores. Results of findings and their significance: We found that: 1) SWNTs were effective in inactivating B. anthracis cells, but not effective to B. anthracis spores. However, SWNTs can inhibit the biofilm formation from B. anthracis spores in suspension; 2) SWNTs coupled with oxidizing agents or near infrared laser enhanced its effect to inactivate B. anthracis spores; 3) Multi-well CNTs (MWNTS)-coated surfaces enhanced the attachment of B. anthracis spores, when in combination with natural peptide nisin, it can inhibit the biofilm formation from B. anthrancis spores. Overall, the project has resulted in 8 peer reviewed journal publications, 3 M.S. graduate student theses, a number of national conference presentations and student presentations/awards at college, university and local/regional conferences.

Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

Received		Paper
08/06/2013	8.00	Marquita Lilly, Xiuli Dong, Eric McCoy, Liju Yang. Inactivation of Bacillus Anthracis Spores By Single Walled Carbon Nanotubes Coupled with Oxidizing Antimicrobial Chemicals, Environmental Science & Technology, (12 2012): 13417. doi: 10.1021/es303955k
08/06/2013	9.00	XIULI DONG, YONGAN TANG, MARQUITA LILLY, KAMAL AFERCHICH, LIJU YANG. ANTIMICROBIAL EFFECTS OF CARBON NANOTUBES, Nano LIFE, (12 2012): 1230012. doi: 10.1142/S1793984412300129
08/16/2012	3.00	Marquita Lilly, Liju Yang, Kamal Aferchich. Effect of Single-walled Carbon Nanotubes on Bacillus Anthracis Cell Growth, Sporulation, and Spore Germination , Journal of Nanoscience and Nanotechnology, (05 2012): 3839. doi:
08/16/2012	7.00	Xiuli Dong, Mei Zhang, Liju Yang1. Attachment of Bacillus anthracis Spores on Multi-walled Carbon Nanotube Coated Surfaces, Journal of Biomedical Nanotechnology, (10 2012): 826. doi:
10/28/2014	13.00	Xiuli Dong, Eric McCoy, Chang Yang, Wei Chen, Ebenezer Addae, Liju Yang. Investigation of Gold/Copper Sulfide Core/Shell Nanoparticles' Antimicrobial Activity to Bacterial Spores and Cells , Journal of Biological Engineering, (08 2014): 11. doi:
10/28/2014	15.00	Xiuli Dong, Yongan Tang, Marvin Wu, Branislav Vlahovic, Liju Yang. Dual effects of single-walled carbon nanotubes coupled with near-infrared radiation on Bacillus anthracis spores: inactivates spores and stimulates the germination of surviving spores, Journal of Biological Engineering, (12 2013): 0. doi: 10.1186/1754-1611-7-19
TOTAL:	**6**	

Number of Papers published in peer-reviewed journals:

(b) Papers published in non-peer-reviewed journals (N/A for none)

Received	Paper

TOTAL:

Number of Papers published in non peer-reviewed journals:

(c) Presentations

1. "Effect of Gold/Copper Sulfide Core/Shell Nanoparticles on Bacillus Anthracis Spores and Cells", E. Addae1, M. Lilly1, E. McCoy1, C. Yang2, W. Chen2, L. Yang1*, 127th North Carolina American Chemical Society Conference, Nov. 1, 2013, Poster presentation,

2. Ebenezer Addae1, Marquita Lilly1, Eric McCoy1, Chang Yang2, Wei Chen2, Liju Yang1. 2013. Effect of Gold/Copper Sulfide Core/Shell Nanoparticles on Bacillus Anthracis Spores. The Institute of Biological Engineering (IBE) 2013 Annual Meeting, March 7-9, Cary, NC. (poster)

3. Liju Yang. 2013.Carbon Nanotubes Interfacing Bacillus anthracis Spores The Institute of Biological Engineering (IBE) 2013 Annual Meeting, March 7-9, Cary, NC. (Oral)

4. Xiuli Dong1, Yongan Tang2, Marvin Wu2, Branislav Vlahovic2, Liju Yang. 2013. Dual Effects of Single-Walled Carbon Nanotubes (SWCNTs) Coupled with Near-Infrared Radiation on Bacillus Anthracis Spores: Inactivates Spores and Stimulates the Germination of Surviving Spores. The Institute of Biological Engineering (IBE) 2013 Annual Meeting, March 7-9, Cary, NC.

5. Xiuli Dong1, Yongan Tang2, Marvin Wu2, Branislav Vlahovic2, Liju Yang1. 2012. Dual effects of Single Walled Carbon Nanotubes Coupled with Near-Infrared Radiation on Bacillus Anthracis Spores: Inactivates Spores and Stimulates the Germination of the Surviving Spores. The Southeastern Regional Meeting of the American Chemical Society (SERMACS) annual meeting, Nov. 14-17, 2012, Raleigh, NC. (Poster)

6. Ebenezer Addae1, Marquita Lilly1, Eric McCoy1, Chang Yang2, Wei Chen2, Liju Yang1. 2012. Effect of Gold/copper sulfide core/shell nanoparticles on bacillus Anthracis spores. The Southeastern Regional Meeting of the American Chemical Society (SERMACS) annual meeting, Nov. 14-17, 2012, Raleigh, NC. (Poster)

7. Marquita Lilly, Xiuli Dong, Eric McCoy, Liju Yang. 2012. Inactivation of Bacillus anthracis spores by Single Walled Carbon Nanotubes (SWNTs) coupled with chemical agents. The Institute of Biological Engineering (IBE) Annual Meeting, Indianapolis, IN, March 1-3, 2012. (Poster)

8. Xiuli Donga, Mei Zhangb, Liju Yang a, 2012. Attachment of Bacillus anthracis Spores on Carbon Nanotube (CNT) Coated Surfaces. The Institute of Biological Engineering (IBE) Annual Meeting, Indianapolis, IN, March 1-3, 2012. (Oral)

9. Xiuli Donga, Mei Zhangb, Liju Yang. 2011. Bacillus anthracis spores attachment on multi-walled carbon nanotube coated surfaces. Southeast Regional Meeting of American Chemical Society (SERMACS), Richmond, VA, Sept 26-29, 2011 (poster)

10. Marquita Lilly, Liju Yang. 2011. Inactivation of Bacillus anthracis spores by Single Walled Carbon Nanotubes (SWNTs) coupled with chemical agents. Southeast Regional Meeting of American Chemical Society (SERMACS), Richmond, VA, Sept 26-29, 2011 (poster)

11. Marquita Lilly, Kamal Aferchich, Liju Yang*. 2011. Effect of Single-walled Carbon Nanotubes on Bacillus Anthrax Cell Growth, Spore Formation, and Spore Germination, , The 2011 IBE annual conference, March 3-5, 2011, Atlanta, GA. (Oral)

Number of Presentations: 11.00

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received	Paper

TOTAL:

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received	Paper

TOTAL:

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts):

(d) Manuscripts

Received		Paper
08/19/2011	1.00	Kamal Aferchich, Marquita Lilly, Liju Yang. Inhibitory effect of Single-walled Carbon Nanotubes on Bacillus Anthracis Cells and Spores, Journal of Nanoscience and Nanotechnology (08 2011)
08/19/2013	10.00	Xiuli Dong, , Yongan Tang, , Marvin Wu, , Branislav Vlahovic,, Liju Yang. Dual Effects of Single-Walled Carbon Nanotubes (SWCNTs) Coupled with Near-Infrared Radiation on Bacillus anthracis Spores: Inactivates Spores and Stimulates the Germination of Surviving Spores, Journal of Biological Engineering (06 2013)
10/28/2014	11.00	Xiuli Dong, Liju Yang. Inhibitory Effects of Single-walled Carbon Nanotubes on Biofilm Formation from Bacillus anthracis Spores, Biofouling (08 2014)
10/28/2014	12.00	Xiuli Dong, Eric McCoy, Mei Zhang, Liju Yang. Inhibitory effects of nisin-coated multi-walled carbon nanotubes sheet on biofilm formation from Bacillus anthracis spores, Journal of Environmental Sciences (04 2014)
TOTAL:	**4**	

Number of Manuscripts:

Books

Received		Book
TOTAL:		

Received	Book Chapter

TOTAL:

Patents Submitted

Patents Awarded

Awards

Student poster presentation awards:

1. Ebenezer Addae, 127th North Carolina American Chemical Society Conference, Nov. 1, 2013, Poster presentation, Honorable Mention ($50), "Effect of Gold/Copper Sulfide Core/Shell Nanoparticles on Bacillus Anthracis Spores and Cells", E. Addae1, M. Lilly1, E. McCoy1, C. Yang2, W. Chen2, L. Yang1*

2. Kamal Aferchich, NC Section of ACS annual meeting, graduate poster award, Honorable mention #1 st place ($50), NCSU, Sept. 29, 2011. "Effect of single walled carbon nanotubes on Bacillus anthracis cell growth and spore formation", Kamal Afrechich, Liju Yang.

3. Kamal Aferchich, College of Science and Technology (CST) research symposium, graduate oral presentation, 1 st place ($100), NCCU, April 13, 2012. "Effect of signle walled carbon nanotubes on Bacillus anthracis cell growth and spore formation", Kamal Afrechich, Liju Yang.

4. Marquita Lilliy, College of Science and Technology (CST) research symposium, graduate oral presentation, 3rd place ($50), NCCU, April 13, 2012. "Single walled carbon nanotubes coupled with chemical for inactivation of Bacillus anthracis spores", Marquita Lilly, Liju Yang.

5. Ebenezer Addae, Graduate student presentation winner at the International Society of Pharmaceutical Engineers (ISPE) NCCU chapter Research Day, NCCU, April 3, 2013 ($25). "Effect of Gold/Copper Sulphide (Au/CuS) Nanoparticles on Bacillus anthracis spores and cells" Ebenezer Addae, Liju Yang.

6. Ebenezer Addae, College of Arts and Science (CAS) research symposium, graduate oral presentation, 2rd place ($75), NCCU, April 20, 2013. "Effect of Gold/Copper Sulfide Core/Shell Nanoparticles on Bacillus Anthracis Spores and Cells", E. Addae1, M. Lilly1, E. McCoy1, C. Yang2, W. Chen2, L. Yang1*

Graduate Students

NAME	PERCENT_SUPPORTED	Discipline
Ebenezer Addae	1.00	
Maquita Lilly	1.00	
Kamal Afechich	1.00	
FTE Equivalent:	**3.00**	
Total Number:	**3**	

Names of Post Doctorates

NAME	PERCENT_SUPPORTED
Xiuli Dong	1.00
FTE Equivalent:	**1.00**
Total Number:	**1**

Names of Faculty Supported

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Names of Under Graduate students supported

NAME	PERCENT_SUPPORTED	Discipline
Courtney Spearman	0.50	Pharmacuetical Sciences
Eric McCoy	0.25	Pharmacuetical Sciences
Jessica McKoy	0.25	Pharmacuetical Sciences
William Tyson	0.50	Pharmacuetical Sciences
FTE Equivalent:	**1.50**	
Total Number:	**4**	

Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period:	1.00
The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:.....	1.00
The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:.....	0.00
Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):.....	0.00
Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering:.....	0.00
The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense	0.00
The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields:	0.00

Names of Personnel receiving masters degrees

NAME	
Ebenezer Addae	
Marquita Lilly	
Kamal Afechich	
Total Number:	**3**

Names of personnel receiving PHDs

NAME
Total Number:

Names of other research staff

NAME	PERCENT_SUPPORTED

FTE Equivalent:
Total Number:

Sub Contractors (DD882)

Inventions (DD882)

Scientific Progress

See attachment

Technology Transfer

N/A

FINAL REPORT

Project title: Inactivation of *Bacillus Anthracis* Spores Using Carbon Nanotubes.

Sponsor Agency: US Department of the Army

Grant#: W911NF-10-1-0160

6/1/2010 to 5/31/2014

Abstract

The Specific Aims of the project were to investigate: 1) the effect of single-walled carbon nanotubes (SWNTs) with different surface groups on the inactivation of *B. anthracis* spores; 2) the effect of SWNTs coupled with near-infrared (NIR) on *B. anthracis* spores; 3) the effect of SWNTs in combination with antimicrobial chemicals on inactivation of *B. anthracis* spores; 4) the effect of CNTs coated surfaces on the inactivation of *B. anthracis* spores; 5) the mechanisms of the inactivation effects of SWNTs to *B. anthracis* spores. **Results of findings and their significance:** We found that: 1) SWNTs were effective in inactivating *B. anthracis* cells, but not effective to *B. anthracis* spores. However, SWNTs can inhibit the biofilm formation from *B. anthracis* spores in suspension; 2) SWNTs coupled with oxidizing agents or near infrared laser enhanced its effect to inactivate *B. anthracis* spores; 3) Multi-well CNTs (MWNTS)-coated surfaces enhanced the attachment of *B. anthracis* spores, when in combination with natural peptide nisin, it can inhibit the biofilm formation from *B. anthrancis* spores. Overall, the project has resulted in 8 peer reviewed journal publications, 3 M.S. graduate student theses, a number of national conference presentations and student presentations/awards at college, university and local/regional conferences.

Results of Findings

In this project, we have successfully completed all the Research Aims and obtained satisfactory results. Findings include the following: 1) ***SWNTs were effective in inactivating B. anthracis cells at concentrations higher than 100 µg/ml. However, SWNTs did not show inactivation effect on B. anthracis spores at concentration up to 300 µg/ml.*** In cell inactivation, the efficiency was buffer-dependent, which SWNTs did not show inactivation effect in PBS buffer, while it showed inactivation effect in DI water and 0.9% NaCl. The inactivation effect was also SWNTs' concentration- and treatment time – dependent. The inactivation mechanism was likely due direct contact between SWNTs and bacterial cells, and resulting cell membrane damages. In addition, SWNTs treatment did not induce sporulation of *B. anthracis*. [Aferichich, et al. 2012]. ***2) SWNTs in combination with oxidizing agents enhanced the inactivation effect to B. anthracis spores***. We investigated the sporicidal effects of SWNTs combined with oxidizing antimicrobial chemicals, H_2O_2 and NaOCl, on *B. anthracis* spores. The results indicated that treatment with SWCNTs alone exhibited little sporicidal effect on *B. anthracis* spores, while treatment of H_2O_2 or NaOCl alone showed moderate sporicidal effect. The combination treatment of SWCNTs (100 µg/mL) with H_2O_2 (1.5%) or NaOCl (0.25%) exhibited much stronger sporicidal effect on

the spores compared to treatment of H_2O_2 or NaOCl alone at the same concentrations, which doubled the log reduction of viable spore number (~3.3 log vs. ~1.6 log). Such enhanced sporicidal efficiency was due to the synergistic effect contributed by the two individual antimicrobial mechanisms of SWCNTs and the oxidizing antimicrobial chemicals. [Lilly et al. 2012]. ***3) carbon nanotube (CNT)-coated surfaces enhanced the attachment but did not inactivation of B. anthracis spores.*** We tested two types of CNT-coated surfaces—CNT forest and CNT sheet. Both surfaces had higher hydrophobicity than their respective substrates and enhanced the attachment of *B. anthracis* spores, but the CNT-coatings did not inactivate the attached spores. The results indicated CNT-coated surfaces had their potential applications as being *Bacillus* spore adsorbents for removing spores from fluids without releasing internal contents to the environment. [Dong, et al. 2012]. ***4) SWCNTs coupled with near infrared (NIR) laser treatment for inactivation of B. anthracis spores in suspensions***. We found that the treatment of 10 μg/mL SWCNTs coupled with 20 min NIR significantly improved the antimicrobial effect by doubling the percentage of viable spore number reduction compared with SWCNTs alone treatment (88% vs. 42%). The treatment also stimulated the germination of surviving spores. Molecular level examination showed that SWCNTs-NIR changed the expression level of several germination, regulatory, and virulence related genes. [Dong et al. 2013]. ***5) Multi-walled CNTs (MWCNTs)-coated surface in combination with natural peptide-nisin inhibited biofilm formation from B. anthracis spores***. We found that nisin coating on MWCNT sheets was highly effective in reducing the germination of attached spores by 8.39 fold, and inhibiting the biofilm formation by 94.6% compared to that on uncoated MWCNTs sheet. The results of this study demonstrated CNTs in combination with nisin had a potential in producing anti-biofilm formation surfaces. [Dong et al. 2014]. ***6) SWNTs inhibition biofilm formation from B. anthracis spores in suspension.*** We studied the inhibitory effect of SWNTs on the biofilm formation from *B. anthracis* spores. Although the presence of 50 to 100 μg/mL of SWCNTs in the suspension increased the spores' attachment in the wells of 96-well plates, the presence of 200 μg/mL of SWCNTs in the germination solution substantially decreased the germination percentage of the attached spores by 93.14%, completely inhibited subsequent biofilm formation. It is also found that the treatment of SWCNTs in the earlier stages of biofilm was more effective than those treatment given at late stages. Mature biofilms were highly resistant to SWCNTs treatment. [Dong and Yang, 2014]. ***7) The fund also partially supported us to explore other nanoparticles for inactivation of B. anthracis cells and spores.*** We investigated the effect of Au/CuS core/shell nanoparticles for inactivating *B. anthracis* cells and spores. It was found that Au/CuS NPs were highly efficient in inactivating *B. anthracis* cells, but not effective to the spores. Treatment with NPs at ~0.83 μM for 30 min achieved a 7 log reduction in viable cells. The antimicrobial effect was both NPs concentration and treatment time dependent. SEM imaging and the efflux of DNA test demonstrated the damage of cell membrane after NPs treatment, yet further research is necessary to fully understand the precise inactivation mechanism. [Addae et al. 2014].

List of publications:

1. Xiuli Dong, **Liju Yang***. 2014**.** Inhibitory Effects of Single-walled Carbon Nanotubes on Biofilm Formation from *Bacillus anthracis Spores**.*** *Biofouling*, Accepted

2. Xiuli Dong[a], Eric McCoy[a], Mei Zhang[b], **Liju Yang[a]***, 2014. Inhibitory effects of nisin-coated multi-walled carbon nanotubes sheet on biofilm formation from *Bacillus anthracis* spores. Accepted, *Journal of Environmental Sciences.*

3. Ebenezer Addae[1], Xiuli Dong[1], Eric McCoy[1], Chang Yang[2], Wei Chen[2], **Liju Yang[1]***. 2014. Investigation of Antimicrobial Activity of Photothermal Therapeutic Gold/Copper Sulfide Core/Shell Nanoparticles to Bacterial Spores and Cells. *Journal of Biological Engineering*, 8:11. doi:10.1186/1754-1611-8-11.

4. Xiuli Dong[1], Yongan Tang[2], Marvin Wu[2], Branislav Vlahovic[2] and **Liju Yang**[1*], 2013. Dual effects of single-walled carbon nanotubes coupled with near-infrared radiation on *Bacillus anthracis* spores: inactivates spores and stimulates the germination of surviving spores *Journal of Biological Engineering* 2013, **7**:19, doi:10.1186/1754-1611-7-19.

5. Marquita Lilly, Xiuli Dong, Eric McCoy, **Liju Yang***. 2012. Inactivation of Bacillus Anthracis spore by single-walled carbon nanotubes coupled with oxidizing antimicrobial chemicals. *Environ. Sci. Tech*. 46, 13417-13424. (Dec)

6. Xiuli Dong, Yongan Tang, Marquita Lilly, Kamal Aferchich, **Liju Yang***. 2012. Antimicrobial effects of carbon nanotubes. *NanoLife*, 2(4): 1230012.

7. Xiuli Dong, Mei Zhang, **Liju Yang*.** 2012. Attachment of Bacillus Anthracis Spores on Multiwalled Carbon Nanotube Coated Surfaces. *Journal of Biomedical Nanotechnology*. 8(5), 826-833. (Oct)

8. Kamal Aferchich, Marquita Lilly, **Liju Yang*.** 2012. Effect of Single-walled Carbon Nanotubes on *Bacillus Anthracis* Cell Growth, Sporulation, and Spore Germination. *Journal of Nanoscience and Nanotechnology*, 12(5), 3839-3847. (May)

M.S. students supported:

Kamal Aferchich (MS), 2009-2012, Graduated May 2012

Maquita Lilly (MS), 2010-2012, Graduated August 2012

Ebenezer Addae (MS), Spring 2012-Fall 2013 (graduated Dec. 2013)

Research Scientist supported:

Xiuli Dong, Ph.D. (Feb.2011-Dec. 2013)